

Soybean response to plant growth regulator herbicides is affected by other postemergence herbicides

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Two studies investigated off-target exposure of soybean to plant growth regulator (PGR) herbicides and determined if simultaneous exposure to PGR herbicides and labeled soybean herbicides increase PGR injury. The PGR herbicides, 2,4-D, clopyralid, and dicamba, as well as dicamba plus the auxin transport inhibitor diflufenzopyr, were applied to glyphosate-resistant soybean at the V3, V7, and R2 soybean growth stages. Two rates were chosen from previous and preliminary research to approximate threshold rates that would cause a yield reduction so as to distinguish differences in sensitivity between growth stages. All four PGR herbicides caused significant soybean injury, height reduction, and yield loss at one or more application rates and growth stages. Relative to other PGR herbicides, dicamba reduced soybean yield at the lowest rate (a potential rate from residues remaining in improperly cleaned application equipment), followed by clopyralid, with 2,4-D requiring the highest rate to reduce soybean yield (a potential rate from a high level of spray drift). Dicamba and dicamba plus diflufenzopyr were applied at equal fractions of labeled use rates for corn to compare them directly at equivalent levels of off-target movement. Dicamba plus diflufenzopyr caused less injury and yield loss than dicamba applied alone. In a second study, the highest labeled soybean use rates of glyphosate, imazethapyr, imazamox, and fomesafen were applied alone and in combination with the highest rate of dicamba used in the first study (1% of a labeled use rate for corn) at the V3 and V7 stages. Dicamba demonstrated synergistic interactions with imazamox, imazethapyr, and fomesafen (but not with glyphosate) to further reduce yield under some circumstances, especially when applied at the V7 stage. Several treatments that included dicamba reduced soybean seed weight when applied at either the V3 or V7 stage and reduced the number of seeds per pod at the V7 stage.

Nomenclature: clopyralid; 2,4-D; dicamba; diflufenzopyr; fomesafen; glyphosate; imazamox; imazethapyr; corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr. 'Pioneer 94B01RR'.

Key words: Auxinic herbicides, crop injury, herbicide interaction, spray drift, spray tank contamination, synergy.

Plant growth regulator (PGR) herbicides have been widely used in monocotyledonous crops for many years and effectively control a broad spectrum of dicotyledonous weeds. Compared with herbicides with other modes of action, weed resistance to PGR herbicides has been slow to develop (Sterling and Hall 1997), which also increases their appeal. However, soybean is frequently grown in close proximity and often in rotation with monocot crops and is very sensitive to PGR herbicides (Al-Khatib and Peterson 1999; Wax et al. 1969). Reports of soybean injury with symptoms resembling off-target exposure to PGR herbicides have been widespread and recurring (Boerboom 2004; Hager and Nordby 2004), although the cause of injury is not often readily identifiable.

PGR herbicide injury to soybean can result in yield loss, but abnormal foliar symptoms and other developmental abnormalities can occur at rates lower than those required to reduce yield (Auch and Arnold 1978; Wax et al. 1969; Weidenhamer et al. 1989). The PGR herbicides most commonly used in close proximity to soybean fields include 2,4-D, clopyralid, and dicamba. Also, the auxin transport inhibitor diflufenzopyr is used in combination with dicamba and synergizes its activity on dicot weeds (Grossman et al. 2002), although there is no information available on the effect that

the addition of diflufenzopyr to dicamba has on potential soybean injury. Soybean differ in sensitivity between dicamba and 2,4-D. When directly applied at the V3 soybean growth stage, 5.6 g ha⁻¹ of dicamba (1% of a labeled use rate for corn) reduced soybean yield 14 to 34%, whereas 112 g ha⁻¹ of 2,4-D (20% of a labeled use rate for corn) was required to cause a similar reduction (25 to 32%) (Andersen et al. 2004). In addition, off-target movement of dicamba has been reported to result in more soybean injury than 2,4-D. In 1974 in Minnesota, postemergence (POST) use of dicamba and 2,4-D in corn resulted in 68 reports of dicamba injury to soybean and 7 reports of 2,4-D injury to soybean, although 2,4-D was applied to over three times as many hectares of corn as was dicamba (Behrens and Lueschen 1979). Clopyralid also has been shown to cause soybean injury (Bovey and Meyer 1981). A 50% soybean yield reduction was caused by nearly equal rates of clopyralid and dicamba (Smith and Geronimo 1977), although the rates were not reported.

Soybean sensitivity to PGR herbicides varies at different growth stages. Dicamba caused greater yield reductions when exposure occurred at a late vegetative or early reproductive stage, relative to an early vegetative stage (Auch and Arnold 1978; Wax et al. 1969). Reports of soybean sensi-

tivity to 2,4-D are somewhat conflicting, however, with 2,4-D causing the greatest yield response when applied at early vegetative stages (Smith 1965), whereas others report little difference in sensitivity between growth stages (Wax et al. 1969), and yet others report that soybean is more sensitive to 2,4-D as it grows taller (Slife 1956). Little has been reported about soybean sensitivity to clopyralid at different growth stages.

PGR herbicides can unintentionally come in contact with soybean and cause injury through several routes of exposure. Spray particles or volatile active ingredients can drift from neighboring fields. Spray particles can drift in air currents with injury often showing a pattern that follows wind direction (Bode 1987), and many herbicide labels have statements regarding wind speed and drift. Risk of vapor drift depends on the volatility of the herbicide formulation used and can be influenced by environmental factors. Short-chain esters of 2,4-D are very volatile, whereas volatility is lower with long-chain esters and is almost eliminated by amine salts of 2,4-D (Que Hee and Sutherland 1974). Dicamba can volatilize as the free acid and injure soybean even when applied as the dimethylamine salt formulation (Behrens and Lueschen 1979). However, dicamba volatility is reduced by lower temperatures and higher relative humidity. PGR herbicide residues remaining in application equipment after previous applications to a corn crop can also be dislodged when the spray equipment is used in soybean. Labels of products containing dicamba provide information describing how to clean equipment to remove these residues. However, even after following recommended cleaning procedures, dicamba residues can remain in application equipment and be detected in a subsequent spray solution at levels as high as 0.63% of a field use rate in corn (Boerboom 2004).

Previous research has described the effects of PGR herbicides on soybean growth and yield when these herbicides are applied alone. However, it is not currently known if there is an interaction between PGR herbicides and herbicides labeled for POST use in soybean that may increase injury. Data from the National Agricultural Statistics Service (NASS 2002) indicate there has been an increase in the use of POST herbicides in soybean with a concomitant decrease in the use of soil-applied herbicides. The increase in POST herbicide use in soybean increases the potential for herbicides labeled for use in soybean to be present when off-target soybean exposure to PGR herbicides occurs. Dicamba and clopyralid interacted with diclofop to increase yield loss in sunflower (*Helianthus annuus* L.) and lentils (*Lens culinaris* L.), respectively (Derksen 1989). PGR herbicides could also potentially interact with soybean herbicides to increase soybean injury. An interaction is possible if PGR herbicide residues are not cleaned from application equipment or if a PGR herbicide drifts from neighboring fields at or near the time of a herbicide application to soybean. The increased dependence on POST herbicides in soybean increases the necessity to understand how herbicides labeled for use in soybean affect soybean exposed to PGR herbicides.

In this study, PGR herbicides commonly used near soybean fields were applied directly to soybean at reduced rates at different growth stages to determine the effect of off-target PGR herbicide exposure on growth, development, and yield. Soybean herbicides with different modes of action

were included for comparison and to obtain tissue samples for lab analysis (Kelley et al. 2004). In addition, dicamba and several soybean herbicides were applied alone and in combination at two vegetative growth stages to determine whether the presence of POST herbicides labeled for use in soybean would increase the injury caused by dicamba. Dicamba was chosen because of its widespread use in corn and the high number of soybean injury reports attributed to dicamba.

Materials and Methods

Two soybean field experiments were conducted at the Crop Sciences Research and Education Center in Urbana, IL. Fields were planted to corn in previous years and had been chisel plowed each fall after corn harvest. In the spring, fields were tilled with a field cultivator. Glyphosate-resistant soybean variety 'Pioneer 94B01RR' was planted in 0.76-m rows at a rate of 400,000 seeds ha⁻¹ in 2001 and 2002 and 420,000 seeds ha⁻¹ in 2003. Plots were kept weed free with a preemergence application of 2.14 kg ha⁻¹ metolachlor, 44 g ha⁻¹ chlorimuron-ethyl, and 0.27 kg ha⁻¹ metribuzin. All treatments were applied with a CO₂-pressurized backpack sprayer equipped with a 2.3-m-wide handheld boom and five 8003 flat-fan nozzles¹ spaced 46 cm apart that delivered 187 L ha⁻¹ at 221 kPa. The spray boom, narrower than the plot width (3.0 m), was centered over each four-row plot so that the two outside rows were not completely within the spray pattern and acted as a buffer to reduce movement between adjacent plots. Applications were made under mostly calm conditions (wind speed was 4 m s⁻¹ or less) to further reduce drift.

PGR Herbicide Study

To evaluate the effects of current PGR herbicides on soybean development, reduced rates of PGR herbicides were applied in 2001, 2002, and 2003. The soil was a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) in 2001 and 2003 and a Catlin silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls) in 2002. The soil organic matter was 4.8, 4.0, and 4.8%, and the soil pH was 6.6, 6.5, and 6.6, respectively. Soybean was planted on May 30, 2001, June 1, 2002, and May 21, 2003.

Treatments included the diglycolamine salt of dicamba, the sodium salt of dicamba plus the sodium salt of diflufenzopyr, the monoethanolamine salt of clopyralid, the isoocytylester formulation of 2,4-D, imazethapyr as a free acid, the isopropylamine salt of glyphosate, and the sodium salt of fomesafen, each applied at the soybean growth stages and rates presented in Table 1. Imazethapyr, fomesafen, and glyphosate are three of the most commonly used POST herbicides labeled for use in soybean and were included in the experiment so that PGR herbicide injury could be compared with the effects of herbicides labeled for use in soybean. The rates chosen for the PGR herbicides were based on preliminary research (data not shown) to bracket the threshold rate that would cause a yield reduction so as to distinguish any differences in soybean sensitivity to these herbicides at the different growth stages. Less dicamba was included with diflufenzopyr than dicamba applied alone, although these are equal fractions of corn field use rates because diflufenzopyr allows for less dicamba to provide similar weed control

TABLE 1. Soybean injury caused by reduced rates of PGR herbicides applied at the V3, V7, and R2 stages of soybean growth combined across 2001, 2002, and 2003.^{a,b}

Herbicide	Rate	Early soybean injury ^c			Late soybean injury		
		2 WAT			6 WAT	6–7 WAT	4–5 WAT
		V3	V7	R2	V3	V7	R2
	g ae/ha	%					
Dicamba	0.56	37 d	31 e	25 e	16 c	23 cd	26 b
	5.6	50 b	41 c	41 b	29 ab	36 b	38 a
Dicamba + diflufenzopyr	0.2 + 0.08	22 e	17 f	18 f	9 d	12 e	18 c
	2.0 + 0.8	42 cd	38 cd	34 cd	21 bc	28 c	28 b
2,4-D	56	8 f	22 f	19 f	3 e	4 f	7 d
	180	49 bc	52 b	37 bc	30 ab	20 d	25 bc
Clopyralid	2.1	41 cd	32 de	29 de	17 c	26 cd	28 b
	6.6	65 a	64 a	47 a	33 a	61 a	45 a
Imazethapyr	71	4 g	6 g	1 i	0 e	0 g	0 f
Glyphosate	840	1 h	1 h	5 h	0 e	0 g	0 f
Fomesafen	330	8 f	8 g	12 g	0 e	0 g	2 e
Untreated control		0 h	0 h	0 i	0 e	0 g	0 f

^a Abbreviations: PGR, plant growth regulator; WAT, weeks after treatment.

^b Means within a column (treatments applied at the same growth stage) followed by the same letter(s) are not significantly different according to Fisher's Protected LSD (0.05).

^c Visual injury ratings on a scale of 0 to 100% with 0% = no injury and 100% = complete death.

(Grossman et al. 2002). This allows a direct comparison of the effect that the addition of diflufenzopyr to dicamba has on the potential for soybean injury caused by off-target exposure. The fractions of a field use rate in corn represented in this study are 0.1 and 1% for dicamba or dicamba plus diflufenzopyr, 10 and 32% for 2,4-D, and 1 and 3.2% for clopyralid. The higher rates of 2,4-D and clopyralid were not included in 2001 but were added in 2002 and 2003. Because application equipment cleaned using recommended procedures may contain dicamba residues as high as 0.63% of a field use rate (Boerboom 2004), equipment that was not properly cleaned could contain PGR herbicide levels similar to the rates applied in this study of dicamba, dicamba plus diflufenzopyr, or even possibly clopyralid. Also, if a PGR herbicide is applied adjacent to a soybean field at a high spray pressure and with high wind speeds, it is feasible for a PGR herbicide to drift onto soybean at rates as high as the rates of 2,4-D applied in this study. All PGR herbicides were applied with 0.25% (v/v) of a nonionic surfactant.² Glyphosate was applied with ammonium sulfate at 1.9 kg ha⁻¹. Methylated seed oil (MSO)³ and 28% urea ammonium nitrogen (UAN) were each included with imazethapyr at 1.25% (v/v) and with fomesafen at 1.0 and 2.5% (v/v), respectively. Soybean growth stages for PGR herbicide applications were chosen to include a vegetative stage when many herbicides are commonly applied to corn (soybean V3 stage), a growth stage when later rescue treatments for weed escapes in corn are often applied (soybean V7 stage), and a reproductive stage when drift from other sources, such as noncrop and pasture areas, may occur.

The experiment was established as a randomized complete block design with three replications and a factorial arrangement of treatments. Herbicide treatments and growth stages were separate factors. Plots measured 3.0 m wide by 9.1 m in length. All herbicide treatments were applied to soybean in the V3 stage 30 to 37 d after planting (DAP), the V7 stage 43 to 51 DAP, and the R2 stage 61 to 66 DAP. At

the V3 application, soybean were 9 cm tall in 2001, 16 cm tall in 2002, and 22 cm tall in 2003. At the V7 application, soybean were 31 cm tall in 2001, 38 cm tall in 2002, and 44 cm tall in 2003. At the R2 application, soybean were 65 cm tall in 2001, 70 cm tall in 2002, and 72 cm tall in 2003.

Soybean injury and height were recorded 2 wk after treatment (WAT) and again 4 to 7 WAT, depending on the time of application. Visual soybean injury ratings were made on a scale of 0 to 100%, where 0 equals no crop injury and 100 equals complete crop death. Final height was measured when plants reached full height before leaf senescence. Delayed maturity was measured by recording the day on which 95% of the soybean pods in each plot reached a mature color and then comparing that with the day when the untreated control plots matured. Yield was measured by machine harvesting the center two rows from each plot and adjusting the moisture to 13%.

Soybean Herbicide Interaction Study

In 2002 and 2003, four herbicides labeled for use in soybean and a reduced rate of dicamba were applied alone and in combination to evaluate an interaction of soybean herbicides and injury caused by dicamba. The soil was a Flanagan silt loam in 2002, and a Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls) in 2003. The soil organic matter was 4.8 and 5.4% and the soil pH was 6.3 and 6.6, respectively. Soybean was planted on June 3, 2002, and May 21, 2003.

The isopropylamine salt of glyphosate, imazethapyr as a free acid, the ammonium salt of imazamox, and the sodium salt of fomesafen were applied with and without the diglycolamine salt of dicamba, as well as dicamba applied alone, at the growth stages and rates listed in Table 3. The adjuvants and rates included with each herbicide were the same as in the PGR herbicide study. Imazamox was applied with

MSO and 28% UAN, both at 1.25% (v/v). Soybean growth stages for soybean herbicide interaction applications were chosen to include an early vegetative stage (V3) when soybean herbicides are commonly applied and a late vegetative stage (V7) before flowering when rescue treatments for weed escapes are often applied. Drift of a PGR herbicide from outside the soybean field could injure soybean at any stage. However, soybean exposure to a PGR herbicide can occur in the presence of a herbicide labeled for use in soybean only when herbicides are applied to soybean (most commonly during vegetative growth stages). The rate of dicamba chosen to be sufficient to cause a yield reduction but not plant death is equivalent to the highest rate used in the PGR herbicide study and represents a potential rate from improperly cleaned application equipment (Boerboom 2004). The rates of the soybean herbicides were the maximum labeled rates at the time of application. With the highest labeled use rates for soybean herbicides and a rate of dicamba expected to cause a yield reduction, these treatments represent a worst-case scenario to determine whether there is potential for dicamba to interact with soybean herbicides and cause a greater yield loss in their presence than if soybean were exposed to dicamba alone.

The experimental design and number of replications were the same as the PGR herbicide study. Plot size was 3.0 m wide by 11.6 m long in 2002, and 3.0 m wide by 9.1 m long in 2003. Treatments were applied to soybean in the V3 stage 30 to 37 DAP and the V7 stage 45 to 51 DAP. At the V3 application, soybean were 20 cm tall in 2002 and 22 cm tall in 2003. At the V7 application, soybean were 40 cm tall in 2002 and 50 cm tall in 2003. Soybean injury and height were recorded 2 and 6 WAT. Final soybean height, delayed maturity, and grain yield were measured in the same fashion as the PGR herbicide study. Before harvest, 10 plants in a row from the center of each plot were collected and used for yield component analysis.

All data were analyzed with a mixed linear model with Statistical Analysis Systems (SAS 1999). In the PGR herbicide study, data from the 3 yr were combined and years were treated as random effects. In the soybean herbicide interaction study, each year was analyzed separately assuming that 2 yr are not a sufficient random sample to represent the larger population (Carmer et al. 1989). Visual injury data were transformed by arcsine square root before statistical analysis to stabilize variances. Untransformed data are presented with statistical interpretation based on transformed data. Visual injury data for applications at each growth stage were analyzed separately because of the data being collected at different times and under different conditions. Within each factor (herbicide treatment and growth stage), means were separated using Fisher's Protected LSD at the 0.05 level of significance.

Synergistic and antagonistic responses between dicamba and soybean herbicides were determined using the method described by Colby (1967) to calculate expected response of herbicide tank mixtures. Expected response values were calculated by expressing values as a percent of the untreated control, and taking the product of values for each herbicide applied alone included in the combination and dividing by 100. Synergistic or antagonistic responses were determined by significant differences between the expected and observed responses using Fisher's protected LSD at the 0.05 level of

significance. When expected and observed responses are not significantly different, interactions between herbicides in a combination are considered additive.

Results and Discussion

PGR Herbicide Study

By 2 wk after all applications (V3, V7, and R2), soybean had significant foliar injury in response to all PGR herbicides, with more injury as rates increased (Table 1). Dicamba and dicamba plus diflufenzopyr resulted in new trifoliolate leaves that were cupped and crinkled, with the higher rates resulting in smaller leaves and reduced overall growth compared with the lower rates (Figures 1A and 1B). Symptoms caused by 2,4-D included epinasty of leaves and stems and swollen, cracked stems. Clopyralid injury resembled dicamba injury, but there were more thin, strapped leaves with parallel venation and less cupping injury (Figures 1C and 1D). Similar symptoms have been described previously (Al-Khatib and Peterson 1999; Andersen et al. 2004; Auch and Arnold 1978; Wax et al. 1969; Weidenhamer et al. 1989). Fomesafen caused temporary necrosis of leaf tissue but had no effect on subsequent growth, whereas imazethapyr temporarily stunted plant growth. Glyphosate caused no visible plant injury, except that the youngest leaves temporarily exhibited chlorosis after the R2 application. The terminal growing point was killed by the higher rate of dicamba or clopyralid at all application timings, by the higher rate of dicamba plus diflufenzopyr at V3 and V7, and by the lower rate of clopyralid at the V7 application. Two WAT at all growth stages, soybean plants treated with the higher rates of PGR herbicides were 10 to 50% shorter than the untreated control (data not shown). Soybean treated with the higher rates of 2,4-D or clopyralid at all growth stages showed little to no increase in height during the 2 wk after treatment.

By 4 to 7 WAT, soybean had recovered from injury caused by fomesafen, imazethapyr, and glyphosate, and injury caused by most PGR herbicides had decreased (Table 1). Soybean treated with the lower rate of dicamba at V3 and the lower rates of dicamba plus diflufenzopyr and 2,4-D at both V3 and V7 showed signs of recovery (emerging trifoliolate leaves lacked injury symptoms). Injury symptoms from both rates of clopyralid and the higher rates of the other PGR herbicides remained more persistent, with the most severe injury from the high rate of clopyralid applied at V7.

All PGR herbicides resulted in a significant reduction in final soybean height, except for the lower rate of dicamba applied at R2 and the lower rate of dicamba plus diflufenzopyr at V7 and R2. Treatments that resulted in the death of the terminal growing point (as mentioned previously) stimulated development of lateral branches for subsequent growth, yet resulted in a 16 to 42% reduction in final height (Table 2). Although the higher rate of 2,4-D did not kill the terminal growing point, it resulted in soybean with severe stem epinasty and an 18 to 25% reduction in final height. The greatest height reductions resulted from the higher rates of all four PGR herbicide treatments at V7, with the higher rate of clopyralid reducing height the most.

Several PGR herbicide treatments caused significant delays in soybean maturity (Table 2). Except for the R2 ap-

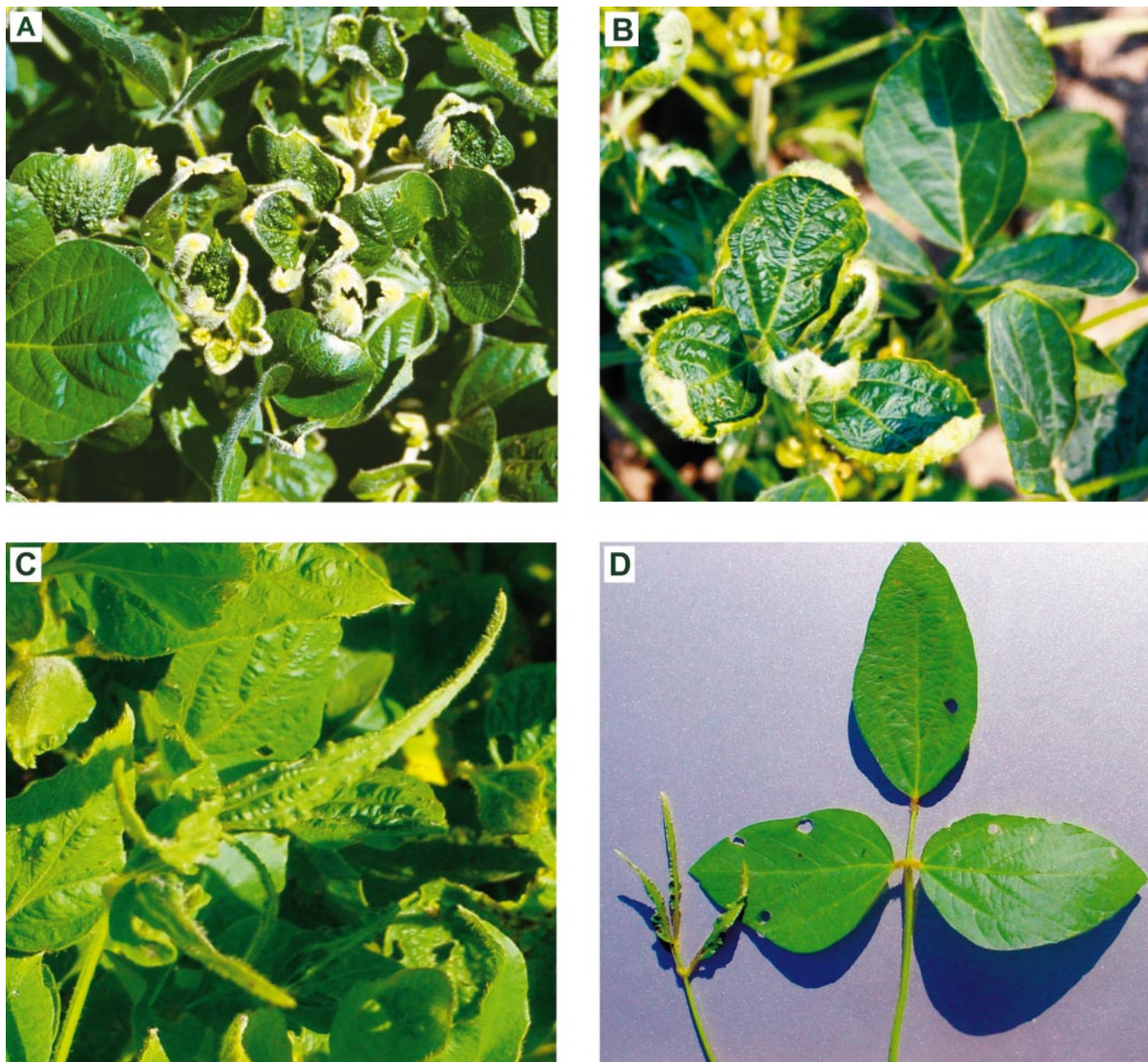


FIGURE 1. Foliar leaf abnormalities caused by plant growth regulator (PGR) herbicides in soybean. (A) and (B) treated with dicamba at 5.6 g ha^{-1} , (C) and (D) treated with clopyralid at 2.1 g ha^{-1} . (D) Clopyralid injured trifoliolate on the left and untreated trifoliolate on the right.

plication at the lower rates, all dicamba and clopyralid treatments resulted in delayed maturity, whereas only the V3 and V7 applications of the higher rate of dicamba plus diflufenzopyr caused a delay. Maturity was delayed by 2,4-D at both rates applied at R2 and the higher rate applied at V7. Injury from higher rates of dicamba, 2,4-D, and clopyralid applied during flowering development (R2) caused the greatest delay in maturity.

Notwithstanding significant injury and reduced height, many PGR herbicide treatments did not result in yield reductions (Table 2). Dicamba plus diflufenzopyr reduced yield by 8% when the higher rate was applied at V3, whereas dicamba reduced yield from 6 to 12% after application of the higher rate at all growth stages and the lower rate at V3. Yield was reduced by 15 to 25% from the higher rates

of 2,4-D applied at all growth stages, and clopyralid reduced yield by 9 to 48% from the higher rate applied at all stages and the lower rate applied at V3. The higher rate of clopyralid applied at V7 resulted in the lowest yield (Table 2). Imazethapyr applied at V7 also reduced yield by 7%.

The growth stages at which soybean were most sensitive to height or yield reductions (or both) varied among the herbicides (Table 2). The highest rate of clopyralid applied at V7 reduced height and yield more than the same rate applied at the other growth stages. The highest rate of dicamba applied at V7 also reduced height more than the same rate applied at the other growth stages, but dicamba did not have a similar effect on yield. Previous research (Auch and Arnold 1978; Wax et al. 1969) showed that dicamba caused greater injury and yield reduction when ap-

TABLE 2. Soybean yield, rate of maturity, and height in response to application of reduced rates of PGR herbicides applied at the V3, V7 and R2 stages of soybean growth combined across 2001, 2002, and 2003.^{a,b}

Herbicide	Rate g ae/ha	Soybean yield ^c kg ha ⁻¹			Maturity delay ^d			Height ^e		
		V3	V7	R2	V3	V7	R2	V3	V7	R2
Dicamba	0.56	2,820 bc	3,120 a	3,270 a	4 bc	3 abc	0 d	88 c	87 c	97 a
	5.6	2,830 bc	2,660 de	2,800 de	7 a	4 ab	9 a	77 e	65 f	81 c
Dicamba + diflufenzopyr	0.2 + 0.08	2,970 ab	3,080 ab	3,040 bc	1 d	0 d	1 cd	95 b	98 ab	97 a
	2.0 + 0.8	2,790 bc	3,000 abc	3,150 ab	5 ab	4 ab	1 cd	84 cd	76 e	88 b
2,4-D	56	2,850 ab	2,890 bc	2,970 bcd	2 cd	2 bcd	4 b	95 b	88 c	87 b
	180	2,270 d	2,520 e	2,570 e	1 d	3 abc	8 a	82 de	75 e	79 c
Clopyralid	2.1	2,740 bc	2,940 abc	3,180 ab	6 ab	5 a	0 d	86 cd	81 d	92 b
	6.6	2,580 c	1,560 f	2,670 e	6 ab	2 bcd	8 a	71 f	58 g	72 d
Imazethapyr	71	2,890 ab	2,820 cd	2,980 bcd	1 d	2 bcd	1 cd	98 ab	96 b	99 a
Glyphosate	840	3,040 a	2,900 abc	3,110 abc	1 d	1 cd	3 cd	98 ab	99 ab	99 a
Fomesafen	330	2,920 ab	2,960 abc	2,930 cd	2 cd	2 bcd	0 d	98 ab	98 ab	98 a
Untreated control		3,020 a	3,020 ab	3,020 bc	0 d	0 d	0 d	100 a	100 a	100 a
LSD (0.05) ^f			270			3			6	
LSD (0.05) ^g			220			3			4	

^a Abbreviation: PGR, plant growth regulator.^b Means within a column (treatments applied at the same growth stage) followed by the same letter(s) are not significantly different according to Fisher's Protected LSD (0.05).^c Measured by harvesting the center two rows from each plot and adjusting moisture to 13%.^d Measured by recording the day when 95% of the pods reached a mature color and comparing with the untreated control.^e Measured when plants reached full height before leaf senescence. Final height of untreated plants was 102 cm.^f Between growth stages for the same herbicide treatment (only the higher rates of 2,4-D and clopyralid—applied only in 2002 and 2003).^g Between growth stages for the same herbicide treatment (only those treatments applied in all years).

TABLE 3. Soybean injury caused by combinations of dicamba and herbicides labeled for use in soybean applied at the V3 and V7 stages of soybean growth.^a

Growth stage	Herbicide	Rate	Early-season injury ^b		Late-season injury	
			2 WAT ^c		6 WAT	
			2002	2003	2002	2003
		g ae/ha	%			
V3	Glyphosate	1,270	0 d	0 e	0 c	0 d
	Imazethapyr	71	2 d	3 d	0 c	0 d
	Imazamox	44	3 cd	5 d	1 c	0 d
	Fomesafen	330	5 c	5 d	0 c	0 d
	Dicamba	5.6	42 b	32 c	27 b	23 c
	Glyphosate + dicamba	1,270 + 5.6	47 b	33 bc	30 b	25 bc
	Imazethapyr + dicamba	71 + 5.6	50 ab ^d	42 ab*	29 b	28 ab*
	Imazamox + dicamba	44 + 5.6	53 ab*	43 a*	32 ab	30 a*
	Fomesafen + dicamba	330 + 5.6	60 a*	48 a*	37 a*	30 a*
	Untreated control		0 d	0 e	0 c	0 d
V7	Glyphosate	1,270	0 c	2 e	0 e	0 c
	Imazethapyr	71	0 c	5 d	0 e	0 c
	Imazamox	44	2 c	5 d	0 e	0 c
	Fomesafen	330	5 b	7 d	0 e	0 c
	Dicamba	5.6	27 a	28 c	30 d	37 b
	Glyphosate + dicamba	1,270 + 5.6	30 a	35 bc*	33 c	43 b*
	Imazethapyr + dicamba	71 + 5.6	37 a*	40 ab*	40 a*	50 a*
	Imazamox + dicamba	44 + 5.6	33 a*	40 ab*	42 a*	52 a*
	Fomesafen + dicamba	330 + 5.6	37 a*	48 a*	38 b*	57 a*
	Untreated control		0 c	0 e	0 e	0 c

^a Means of treatments applied in the same year at the same growth stage followed by the same letter(s) are not significantly different according to Fisher's Protected LSD (0.05).

^b Visual injury ratings on a scale of 0 to 100% with 0% = no injury and 100% = complete death.

^c Abbreviation: WAT, weeks after treatment.

^d * Indicates significant synergistic interaction at the 0.05 level.

plied near the R2 or V7 stage, relative to the V3 stage. The higher rate of 2,4-D resulted in the lowest yield from the V3 application, significantly lower than the R2 application at $P < 0.05$, and the V7 application at $P < 0.1$. Smith (1965) also reported lower yields after 2,4-D was applied to soybean at an early vegetative stage compared with a reproductive stage. Many of the applications of dicamba plus diflufenzopyr resulted in reduced crop injury (Table 1) and greater height and yield (Table 2) compared with an equal fraction of a field use rate of dicamba in corn. The addition of diflufenzopyr to dicamba, which allows a reduction in the amount of dicamba necessary to achieve adequate weed control, may reduce injury caused by off-target exposure to dicamba-containing products.

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By 2 wk after the V3 and V7 applications, treatments that included dicamba caused a considerable amount of injury (Table 3), including death of the terminal growing point and leaf cupping symptoms (Figures 1A and 1B). When applied alone, fomesafen caused temporary leaf necrosis but had no effect on subsequent growth, imazethapyr and imazamox temporarily stunted plant growth, and glyphosate caused no significant plant injury. Imazethapyr, imazamox, and fomesafen all demonstrated synergistic interactions with dicamba, increasing soybean injury at 2 wk after both application timings in both years, and glyphosate had a similar interaction with dicamba after the V7 application in 2003 (Table 3).

By 6 wk after both application timings, dicamba-treated soybean were still showing foliar leaf cupping symptoms and were reduced in height (Table 3). When applied at V3, there were synergistic interactions between the following soybean herbicides and dicamba to increase soybean injury 6 WAT: fomesafen in both years and imazethapyr and imazamox in 2003. When applied at V7, there were synergistic interactions between the following herbicides and dicamba to increase injury 6 WAT: imazethapyr, imazamox, and fomesafen in both years, and glyphosate in 2003. Dicamba-treated plants (alone or with another herbicide) failed to achieve canopy closure and all leaves that emerged after application exhibited cupping injury symptoms, with leaves that were smaller than leaves from plants not treated with dicamba (data not shown).

All treatments that included dicamba caused a significant reduction in final soybean height, whereas herbicides labeled for use in soybean did not reduce final height in the absence of dicamba (Table 4). Dicamba applied alone at V3 reduced final soybean height by 21 to 22%, and when applied at V7, dicamba applied alone reduced height by 25 to 28%. When applied at the V7 application both years, there were synergistic interactions between dicamba and imazamox or fomesafen to further reduce final soybean height, whereas similar interactions occurred with dicamba plus imazethapyr in 2002 and dicamba plus glyphosate in 2003.

Dicamba treatments had a significant effect on the rate of soybean maturity, but the effect varied between 2002 and 2003 (Table 4). Most treatments containing dicamba re-

TABLE 4. Soybean yield, rate of maturity, and height in response to combinations of dicamba and herbicides labeled for use in soybean applied at the V3 and V7 stages of soybean growth.^a

Growth stage	Herbicide	Rate g ae/ha	Soybean yield ^b kg ha ⁻¹		Delayed maturity ^c		Final height ^d	
			2002	2003	2002	2003	2002	2003
V3	Glyphosate Imazethapyr Imazamox Fomesafen Dicamba	1,270 71 44 330 5.6	3,280 a 3,320 a 2,870 bcd 3,190 ab 2,690 cde	3,370 abc 3,130 bcd 3,390 ab 3,470 ab 2,720 ef	0 b 0 b 0 b 0 b 5 a	0 b 0 b 0 b 1 b 6 a	104 a 104 a 96 a 103 a 78 b	98 a 95 a 94 a 94 a 79 b
	Glyphosate + dicamba Imazethapyr + dicamba Imazamox + dicamba Fomesafen + dicamba Untreated	1,270 + 5.6 71 + 5.6 44 + 5.6 330 + 5.6	2,730 cd 2,950 abc 2,540 de 2,340 e 3,160 ab	3,010 cde 2,440 f 2,930 de 2,440 f 3,490 a	4 a 3 a 4 a 2 ab 0 b	7 a 6 a 7 a 7 a 0 b	80 b 76 b 73 b 71 b 100 a	79 b 73 b 77 b 74 b 100 a
	Glyphosate Imazethapyr Imazamox Fomesafen Dicamba	1,270 71 44 330 5.6	3,200 a 3,160 ab 3,010 abc 3,280 a 2,790 c	3,270 a 3,340 a 3,400 a 3,330 a 2,500 b	1 a 1 a 0 a 1 a -4 b	0 b 0 b 0 b 1 b 5 a	99 a 102 a 102 a 100 a 75 b	98 a 99 a 94 a 96 a 72 b
	Glyphosate + dicamba Imazethapyr + dicamba Imazamox + dicamba Fomesafen + dicamba Untreated LSD (0.05) ^f	1,270 + 5.6 71 + 5.6 44 + 5.6 330 + 5.6	2,580 d 2,060 e* 1,970 e* 2,070 e* 3,160 ab 390	2,300 bc 2,200 bc 2,110 bc 2,060 c* 3,490 a 410	-4 b -4 b -4 b -4 b 0 a 3	6 a 5 a 6 a 6 a 0 b NS	73 bc 64 cd* 63 d* 63 d* 100 a 8	63 c* 65 bc 61 c* 60 c* 100 a 8

^a Means of treatments applied in the same year at the same growth stage followed by the same letter(s) are not significantly different according to Fisher's Protected LSD (0.05).^b Measured by harvesting the center two rows from each plot and adjusting moisture to 13%.^c Measured by recording the day when 95% of the pods reached a mature color and comparing with the untreated control. NS, not significant.^d Measured when plants reached full height before leaf senescence. Final heights of untreated plants were 81 cm in 2002 and 111 cm in 2003.^e * Indicates significant synergistic interaction at the 0.05 level.^f Between growth stages for the same herbicide treatment.

TABLE 5. Rainfall before and after treatments of the soybean herbicide interaction study.^a

	2002		2003	
	V3	V7	V3	V7
	mm			
1 MBT ^b	59	1	64	151
1 WAT	0	35	6	0
2 WAT	0.3	24	106	52
3 WAT	35	0	0	5

^a Illinois Climate Network Data, Illinois State Water Survey for Champaign, IL.

^b Abbreviations: MBT, month before treatment, WAT, week after treatment.

sulted in delayed maturity. However, applications at V7 in 2002 resulted in earlier maturity. The late-season injury (Table 3) and final height data (Table 4) indicate that dicamba applied at V7 was potentially more damaging to soybean than at V3. Precipitation received before and after the V7 application in 2002 was less favorable than in 2003, as illustrated by the rainfall data in Table 5. These plants in 2002 had received only 1 mm of rainfall during the entire month before treatment, resulting in drought-stressed plants that were further stressed by dicamba. Soybean received rainfall after herbicide treatment (Table 5), but it is likely that the addition of dicamba injury and drought stress at application caused enough damage to result in premature senescence (Table 4), although this did not appreciably alter the response of soybean height or yield to dicamba injury between the 2 yr. Notably, the addition of another herbicide to dicamba did not affect maturity.

Yield results also show a significant impact of the presence of herbicides labeled for use in soybean on dicamba injury. Less favorable rainfall in 2002 resulted in lower yields, with the untreated control yielding 3,160 kg ha⁻¹ in 2002 compared with 3,490 kg ha⁻¹ in 2003. Soybean yield after applications containing dicamba ranged from 7 to 38% less than the untreated control in 2002 and 14 to 41% less in 2003 (Table 4). Herbicide treatments applied at V7 that resulted in significantly lower yield ($P < 0.05$) than the same treatment applied at V3 included imazamox plus dicamba in both years, imazethapyr plus dicamba in 2002, and glyphosate plus dicamba in 2003. After the V7 application in 2002, there were synergistic interactions between dicamba and imazethapyr, imazamox, or fomesafen to further decrease yield (Table 4), and when applied at V7 in 2003, fomesafen had a similar interaction with dicamba. If the significance level is set at $P < 0.1$, then imazamox applied at V7 in 2002 and fomesafen applied at V3 in 2002 also demonstrated synergistic interactions with dicamba to further decrease yield. Fomesafen plus dicamba resulted in the highest soybean injury rating (Table 3) and the greatest height reduction (Table 4) of all V3 applications in 2002. These results demonstrate that dicamba can cause a greater yield loss in the presence of a herbicide labeled for use in soybean than if there is no other herbicide present, and that among the soybean herbicides included in this study, fomesafen exacerbated yield losses caused by dicamba more than other herbicides.

To determine which growth process was affected to reduce yield, plant samples were collected before harvest for

yield component analysis (Table 6). Seeds per pod were significantly reduced by all applications at V7 that included dicamba in both years. The stress from dicamba may have affected seed development during flowering, which began shortly after the V7 stage. Other treatments that reduced seeds per pod included dicamba applied alone at V3 in 2002, glyphosate plus dicamba at V3 in 2003, and imazethapyr or imazamox applied alone at V7 in 2002. Pods per plant were not significantly affected by dicamba applications (Table 6). Pods per node were reduced in response to dicamba, but nodes per plant were increased (data not shown). Although plants were shorter, they were able to produce sufficient nodes on lateral branches from which pods could develop to offset any reduction in pod set during flowering. The degree of seed and pod development vs. floral abortion is influenced by auxin (Cho et al. 2002). Also, exogenous auxin enhances the growth of different tissues (roots, buds, stems), but only at specific concentrations, with higher concentrations inhibiting growth (Gardner et al. 1985). Therefore, it would be anticipated that PGR herbicides, which overstimulate auxin receptors (Sterling and Hall 1997), would inhibit floral development at a sublethal dose if applied near flowering. Seed weight was significantly reduced by several treatments that included dicamba at both the V3 and V7 stages (Table 6). This appears unusual because seed fill does not begin until late in development, several weeks after the V3 stage. However, decreased seed weight may be due to diminished photosynthetic capacity caused by reduced leaf area, given that dicamba prevented canopy closure and resulted in smaller, malformed leaves (data not shown).

All the herbicides labeled for use in soybean that exacerbated yield losses caused by dicamba are not phytotoxic to soybean due to rapid metabolism of the herbicide (Skipsey et al. 1997; Tecle et al. 1993). However, glyphosate, which did not significantly increase dicamba injury, is not phytotoxic due to an insensitive target site in soybean (Padgett et al. 1995). It may therefore be possible that dicamba injury prevented soybean from metabolizing these herbicides at a sufficient rate to prevent phytotoxicity.

Because the presence of herbicides labeled for use in soybean may affect the level of soybean injury and yield loss caused by dicamba, there is added significance in identifying the route of exposure to a PGR herbicide in a reported case of injury. However, with some reports of soybean symptoms resembling PGR herbicide injury, there is not a readily determined source of PGR herbicide exposure. It could be possible for other sources of stress, such as herbicides with a different mode of action, aphid feeding, or infection by certain soybean viruses, to cause symptoms that are mistaken for PGR herbicide injury (Proost et al. 2004). This makes it difficult to accurately assess the cause of soybean injury, especially because no diagnostic tools are available to conclusively verify that a PGR herbicide is the cause of injury. Another study performed in conjunction with this one explores the development of a diagnostic assay for PGR herbicide injury in soybean based on the expression of auxin-responsive genes (Kelley et al. 2004).

The results of this study reveal differences in the way that soybean responds to PGR herbicides and may influence decisions on their use. Clopyralid caused much greater yield losses at 6.6 g ha⁻¹ when applied at a late-vegetative stage

TABLE 6. Components of soybean yield in response to combinations of dicamba and herbicides labeled for use in soybean applied at the V3 and V7 stages of soybean growth.^a

Growth stage	Herbicide	Rate g ac ha ⁻¹	Seeds per pod		Pods per plant		Seed weight	
			2002	2003	2002	2003	2002	2003
V3	Glyphosate	1,270	2.38 ab	2.47 ab	28 a	28 a	17.03 a	13.84 ab
	Imazethapyr	71	2.42 a	2.52 ab	26 a	31 a	16.27 ab	13.66 ab
	Imazamox	44	2.27 ab	2.51 ab	28 a	37 a	16.79 a	13.75 ab
	Fomesafen	330	2.29 ab	2.53 a	26 a	30 a	16.64 a	13.28 ab
	Dicamba	5.6	2.20 b	2.48 ab	23 a	30 a	14.54 cd	12.69 b
	Glyphosate + dicamba	1,270 + 5.6	2.29 ab	2.35 b	21 a	32 a	13.96 d	13.08 ab
	Imazethapyr + dicamba	71 + 5.6	2.26 ab	2.51 ab	22 a	25 a	14.88 bcd	12.28 b
	Imazamox + dicamba	44 + 5.6	2.29 ab	2.43 ab	25 a	28 a	15.75 abc	13.05 ab
	Fomesafen + dicamba	330 + 5.6	2.27 ab	2.44 ab	25 a	30 a	14.29 cd	14.15 a
	Untreated control		2.41 a	2.53 a	25 a	35 a	16.52 a	14.34 a
V7	Glyphosate	1,270	2.31 ab	2.48 a	31 a	34 a	16.47 abc	13.56 ab
	Imazethapyr	71	2.23 bc	2.43 a	29 a	30 a	16.54 ab	13.88 ab
	Imazamox	44	1.97 d	2.42 a	28 a	31 a	17.12 a	14.44 a
	Fomesafen	330	2.43 a	2.52 a	25 a	28 a	16.17 abc	14.12 ab
	Dicamba	5.6	2.15 bcd	1.90 b	26 a	33 a	14.76 c	12.66 b
	Glyphosate + dicamba	1,270 + 5.6	2.10 cd	2.00 b	26 a	30 a	15.54 bc	12.83 b
	Imazethapyr + dicamba	71 + 5.6	2.05 cd	1.86 b	26 a	29 a	15.23 c	13.41 ab
	Imazamox + dicamba	44 + 5.6	2.00 d	1.86 b	26 a	28 a	15.42 bc	13.41 ab
	Fomesafen + dicamba	330 + 5.6	2.00 d	1.87 b	26 a	26 a	15.37 bc	13.18 ab
	Untreated control		2.41 a	2.53 a	25 a	35 a	16.52 ab	14.34 a
	LSD (0.05) ^b		0.20	0.19	NS ^c	NS	1.47	NS

^a Measured from a sample of 10 plants taken from the center of each plot before harvest. Means of treatments applied in the same year at the same growth stage followed by the same letter(s) are not significantly different according to Fisher's Protected LSD (0.05). Treatments that include more than one herbicide were tested for synergistic interactions using the method described by Colby (1967), but no treatments were significant at the 0.05 level.

^b Between growth stages for the same herbicide treatment.

^c Abbreviation: NS, nonsignificant.

approaching flowering than at an early-vegetative stage or during flowering, whereas dicamba and 2,4-D showed less of a difference among growth stages. Dicamba caused yield losses at the lowest rate, with 2,4-D requiring the highest rate to reduce yield and clopyralid causing yield losses at a rate in between dicamba and 2,4-D. The addition of diflufenzopyr to dicamba, which allows for less dicamba to be applied to maintain adequate weed control, resulted in less of a yield effect than dicamba applied alone at an equal fraction of a field use rate in corn. This indicates that the use of diflufenzopyr may reduce the risk for unintended soybean injury due to dicamba. Results show that soybean responds differently to the various PGR herbicides examined in our study, and an understanding of these differences will allow growers to select a PGR herbicide based on an assessment of their weed management needs and the potential for soybean injury due to off-target movement.

Previous research on the effects of PGR herbicides in soybean has not addressed the impact of the presence of herbicides labeled for use in soybean. However, our results clearly show that the presence of a POST soybean herbicide can significantly exacerbate yield losses caused by off-target dicamba exposure. Dicamba can interact with a soybean herbicide when dicamba herbicide residues are present in application equipment used for soybean. The rate used in this study would not likely be present in application equipment that was cleaned properly, which emphasizes the need to clean application equipment thoroughly after use of a PGR herbicide. Dicamba may also interact in the plant with soybean herbicides when dicamba drifts onto soybean from a neighboring corn field at or near the time of a POST application to soybean, although this type of interaction was not evaluated in this study and may have different consequences than those reported here. Results showed a difference between herbicides that are selective in soybean due to metabolism (imidazolinones and fomesafen) vs. an insensitive target site (glyphosate), which indicates that a reduction in the ability of soybean to metabolize either herbicide may play a role in the interaction between the soybean herbicide and dicamba. Regardless of the mechanism, it is clear that under certain circumstances, the presence of some soybean herbicides can aggravate injury and yield losses caused by dicamba. It would also be of interest to determine if dicamba injury to soybean is affected by other POST herbicides in soybean where selectivity is due to engineered metabolism (e.g., glufosinate-resistant soybean).

Sources of Materials

¹ TeeJet standard flat spray tips, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189-7900.

² Activator-90, nonionic surfactant, a mixture of alkylphenyl hydroxypolyoxyethylene and fatty acids, Loveland Industries Inc., P.O. Box 1289, Greeley, CO 80632-1289.

³ MSO, methylated seed oil and emulsifying surfactants 100%, Loveland Industries Inc., P.O. Box 1289, Greeley, CO 80632-1289.

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